Quantitative Chemical Compositions of Neem (*Azadirachtaindica*) Leaf Aqueous Extracts in Sokoto, Nigeria

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Abstract: **- The aim of the present work was to study the chemical compositions of aqueous extract of Neem (*Azadirachtaindica*) leaf in Sokoto. The results obtained showed the presence of Tannin at1.4%, Oxalate at 1.41mg/100g, Phytate at 6.12mg/100g, Saponin at 22.55%, Cyanogenic glycoside at 2.05mg/100g, Alkaloid at 12.22%, Trypsin inhibitor at 6.25%, Flavonoid at 32.06%, Moisture content at 2.12%, Ash conten at 10.8%, Fibre at 16.20%, Protein at 14.53%, Fat at 5.82%, Carbohydrate at 59.53%, Energy value at 1,399.39Kj/100g, Vitamin A at 19.695mg/L, Vitamin B1 at 3.7mg/L, Vitamin B2 at 3.51mg/L, Vitamin B6 at 358.71mg/L, Vitamin C at 3154mg/L, Vitamin E at 33.98mg/L, Zinc at 0.1572ppm, Sodium at 5.7302ppm, Magnesium at 0.5802ppm, Phosphorus at 8.52ppm, Calcium at 7.2875ppm, Iron at 3.68ppm and Potassium at 9.0ppm**

These results indicated that neem leaf aqueous extracts may serve as a useful ingredient in dietof especially animals and may exhibit immunomodulatory as well as anti-inflammatory, antihyperglycaemic; antiulcer; antimalarial; antifungal; antibacterial; antiviral; antioxidant; antimutagenic and anticarcinogenic characteristics.

Keywords: **Neem leaf aqueous extracts, chemical compositions, Sokoto**

I. INTRODUCTION

Neem also refered to as Nimba/Holy tree/Vembu/Arishtha/Indian neem tree/Indian lilac/Margosa tree is an evergreen tree which is abundantly found in most of tropical and sub-tropical countries including Nigeria. Genus *Azadirachta* has two species as per geographical distribution and *A. indica*is mostly native of Indian and Asian sub-continent. Tree has good growth rate and attains height approximately 15-20m and sometimes even upto 35-40m(Kumar and Navaratnam, 2013).

History reveals the long journey of omnipotent Neem tree from old legendary time to present state defining its role in the sustainability of humankind. In the old annals of the ancient Siddha medicinal system the first medicinal plant described was Neem or Margosa. In ancient immemorial period Neem has been used as adisincentive agent against highly contagious smallpox and other infectious diseases and was also regarded to defend against evil spirits from time (Kumar and Navaratnam, 2013). In old medicinal system medications and applications were compiled over palm leaves and they have been passed on from generations to generations. In the Indus civilization the use of Neem tree is as old as 4500 years during the period of Harappa culture (one among the great civilisation in the world). There the earliest of the documentionmentions the fruits and seeds; oils and leaves; roots and barks for the medical characters that are advantageous (http://www.slideshare.net/imulla/neem-presentation-882503). Writing palm leaf manuscript is among the oldest medium of conserving knowledge in India to store the history of herbal heritage. Centre for Traditional Medicine and Research (CTMR), Chennai. India revealed the medicinal uses of different parts viz, fruits, seeds, leaves, roots, bark etc., of Neem trees. It explains use of neem flower against bile disorders, neem leaves to prevent and treat ulcers and neem bark to brawl against paralysis and CNS disorders (Bandyopadhyay *et al.*, 2004). Old evidences obtained from two great civilisations Harappa and Mohenjo-Daro of ancient world also witnessed that *A. indica*was the prominent herb of therapeutic importance at that time not only in Indian context but in world as well. According to epic of Mahabharata, Nakul and Sahadeva used Neem oils for treatment of wounds in horses and elephants (Bandyopadhyay *et al.*, 2004).

There are mainly two types of metabolites viz.,primary including protein, fatty substance or carbohydrate or sugar derivative and secondary metabolites containing various alkaloids, steroids, flyvanoids, saponins etc. The compounds isolated have been broadly classified into two major groups: isoprenoids and non-isoprenoids. Compounds such as diterpenoids, interpenoids and steroids including azadirone, protoliacin, limionoids, gedunin, vilasinin type of compounds, C-secomeliacins such as azadirachtin, nimbin, salanin and its derivatives comprises the isoprenoids group while non-isoprenoids contains proteins/amino acids, polysaccharides, sulphurous compounds,polyphenolics such as flavonoids, their glycosides, dihydrochalcone, coumarin, tannins, aliphatic compounds, etc(Biswas *et al.*, 2002). Stem bark of neem contains many tannins in condensed form, important ones are tricyclic diterpenoids, NB-11 peptidoglycan, gallic acid,epigallocatechin, gallocatechin, catechin, epicatechin, margolonone and isomargolonone etc. The biological activity of different active principles of Neem exert multi-facet therapeutic effects (Mongkholkhajornsilp *et al.*, 2005; Biu *et al.*, 2009).

They have been used in traditional practices for treating inflammation and infections; fever, skin diseases as well as dental disorders. The leaves as well as its constituents use to exhibit immunonodulatory as well as anti-inflammatory and antihyperglycaemic activities; antiulcer; antimalaria; antifungal; antibacterial; antiviral; antioxidant; antimutageinc as well as anticarcinogenic characteristics (Beuth *et al.,* 2006; Akihisa *et al.,* 2009; Rakib and Hussain 2013). Neem plant preparations are also used by the natural healers for diverse disorders such as leprosy, gastrointestinal problems, malaria, intestinal helminthiasis, tuberculosis, ringworms, skin disorders, boils, epilepsy, fever, respiratory distress, nausea, ulcers and in health industries also (Biswas *et al.,* 2002; Bhowmik *et al.,* 2010; Pankaj *et al.,* 2011). Modern scientists are exploiting more uses of this special tree. Different parts of neem plant such as bark, stems, leaves, fruits, flowers and seeds possess compounds such as flavonoids, tannins, flavonoglycosides, arabinofucoglucanes and others with proven immunomodulatory, antiseptic, diuretic, emmenagogue, febrifuge, antipyretic, antidiabetic, anthelmintic, anti-inflammatory, analgesics, antifeedant, antiviral, antifungal, contraceptive, *in vitro*spermicidal, insecticide, pediculocide, parasiticide, antimutagenic and anti-ulcer properties since antiquity (Al-Samarrai *et al.,* 2012; Delserrone and Nicoletti, 2013; Mukherjee and Sengupta, 2013). In traditional Indian system, Neem leaves were used to cover the patient of chicken pox and the patient was suggested to sleep under neem tree. In an experimental study on rats, neem leaf extract has been reported beneficial in treatment of carbon tetrachloride led liver damage (Mujumdar, 1998).Neem Leaf Meal (NLM) may be useful ingredient in diet of broilers due to its medicinal and nutritional importance (Bonsu *et al.,* 2012). Nimbidiol present in root and bark of neem tree can inhibit intestinal glucosidases thus helpful in control of diabetes (Mukherjee and Sengupta, 2013). In modern era, emphasis should be on control of diseases of human, animals and environment using non-toxic herbal products. By making quantum of research on biological and medicinal properties of neem, some of the herbal products have been prepared but still there is lot of scope in this field for better utilization of this wonder plant (Tiwari *et al.,* 2014).

II. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Sokoto. Sokoto State is geographically located to the North Western part of Nigeria between the longitudes 4^0 8'E and 6^0 54'E, and latitudes 12^0 and 13^0 58N (NPC, 2006). The state falls within two vegetation zones: the Sudan Savannah and Northern Guinea Savannah. The climate is characterized by altering dry and wet seasons with short cold and dry period of harmattan usually accompanied by dust-laden winds and fogs which start from October and last through February. The duration and intensity of annual rain fall ranges from 60-160 days and 635-1000mm (occurring between May to October) respectively. The mean monthly temperature is generally 20-46^0 C, relative humidity ranges from 12-17% with the highest occurring in August (NPC, 2006).

2.2 Preparation and Extraction of Neem (Azadirachtaindica) Leaf Aqueous Extracts

Mature green neem leaves were used for the experiment. The leaves were obtained from ShehuKangiwa Square and a botanist from UDUS herbarium professionally identified the Neem and labelled it (UDUH/ANS/0004). The leaves were rinsed in distilled water, air dried and pulverized. The extract was prepared following the procedure reported by (Sithisarn *et al.,* 2006).

2.3 Phytochemical Determination

Alkaloid, Tannin, Saponin, Flavonoid, Trypsin Inhibitor Activity, Oxalate and Phytate were determined following the standard procedure and protocol as described by Obadoni and Ochuko (2001).

2.4 Mineral Analyses

Mineral was estimated by the used of an Atomic Absorption Spectrophotometer as described by AOAC (2000).

2.5 Proximate Analysis

Procedure including the percentage of moisture content, crude protein, ash contents and crude fiber in the sample were determined by The Association of Official Analytical Chemists methods (AOAC, 1990) while the percentage carbohydrate was determined by the formular as described by Akubugwo *et al.,* (2008)

% carbohydrate= 100 - (% moisture + % crude fibre + % ash + % crude fat+ % crude protein).

2.6Vitamin Analyses

Vitamin A andE was determined spectrophotometrically using a modified standard method of AOAC, (2000), Vitamin C by the method as described by Hussian*et al.,* (2006), Vitamin B 1 by the method as described by Poornima and Ravishankar (2009) and Vitamin B 2 and B 6 by the method as described by Uraku*et al.* (2014).

III. RESULTS

3.1 Neem (Azadirachtaindica) Leaf Aqueous Extract Analyses

3.1.1 Quantitative Phytochemical Analysis of the Neem Leaf Aqueous Extracts

The quantitative phytochemicals of the extract (Table 1) are tannin (1.45%), oxalate (1.41mg/100g), phytate (6.12mg/100g), saponin (22.55%), cyanogenic glycoside (2.05mg/100g), alkaloid (12.22%), trypsin inhibitor (6.25%) and flavonoid (32.06%).

3.1.2 Quantitative Proximate Constituents of the Extracts

The result of the quantitative proximates of the extracts as presented in table 2 are moisture content (2.12%), ash content

(10.8%), fibre (16.20%), carbohydrate (50.53%), protein (14.53%), fat (5.82%) and energy value (1,399.39Kj/100g).

3.1.3 Vitamin Compositions of the Extract

The composition of the vitamins present in the extract was as shown in table 3 was found to be vitamin A (19.695mg/L), vitamin B1 (3.7mg/L), vitamin B2 (3.51mg/L), vitamin B6 (358.71mg/L), vitamin C (3154mg/L) and vitamin E (33.98mg/L).

3.1.4 Mineral Compositions of the Extract

The mineral compositions of the neem leaf extracts are presented in table 4. The determined compositions include zinc (0.1572ppm), sodium (5.7302ppm), magnesium (0.5802ppm), phosphorus (8.52ppm), calcium (7.2875ppm), potassium (9.0ppm) and iron (3.68ppm).

Table 1: Quantitative Phytochemicals of the Extracts

S/N	Parameter	Quantity
1	Tannin	1.4%
2	Oxalate	1.41mg/100g
3	Phytate	6.12mg/100g
4	Saponin	22.55%
5	Cyanogenic glycoside	2.05mg/100g
6	Alkaloid	12.22%
7	Trypsin inhibitor	6.25%
8	Flavonoid	32.06%

Table 2: Quantitative Proximate Constituents of the Extract

S/N	Parameter	Quantity
1	Moisture content	2.12%
2	Ash content	10.8%
3	Fibre	16.20%
4	Protein	14.53%
5	Fat	5.82%
6	Carbohydrate	59.53%
7	Energy value	1,399.39Kj/100g

Table 3: Vitamin Compositions of the Extract

S/N	Parameter	Quantity
1	Vitamin A	19.695mg/L
2	Vitamin B1	3.7mg/L
3	Vitamin B2	3.51mg/L
4	Vitamin B6	358.71mg/L
5	Vitamin C	3154mg/L
6	Vitamin E	33.98mg/L

Table 4: Mineral Compositions of the Extract

S/N	Parameter	Quantity
1	Zinc	0.1572ppm
2	Sodium	5.7302ppm
3	Magnesium	0.5802ppm
4	Phosphorus	8.52ppm
5	Calcium	7.2875ppm
6	Iron	3.68ppm
7	Potassium	9.0ppm

IV. DISCUSSION

In this research, the chemical compositions of aqueous extract of Neem (*Azadirachtaindica*) leaf in Sokoto, Nigeria were studied.

The phytochemical analysis of the extract revealed the presence of tannin, oxalate, phytate, saponin, cyanogenic glycoside, alkaloid, trypsin inhibitor and flavonoid. These findings are in agreement with the results of Madaki*et al.*, (2016) qualitatively. However, almost none of the values obtained by other researchers as well as the present findings agreed with each other quantitatively. The variations could be attributed to the geographical location of the plant, methods of extraction as well as the season in which the leaf was harvested. Generally, phytochemicals are chemical compounds produced by plants which help in the overall maintenance of the health of an organism but are not essential nutrients. These chemicals perform biological functions by acting as antioxidants, physiological agents, antimicrobial agents, and numerous other functions depending on the type of phytochemical present (Egbuna and Ifemeje, 2015). The present findinghas shown the presence of flavonoid (32.06%). The flavonoid has protective effects including anti-inflammatory, anti- oxidant, anti- viral and anti- carcinogenic properties and is relatively less toxic (González *et al.,* 1990). At very high concentration, it chelates metals such as iron and zinc and reduces the absorption of these nutrients. They also inhibit enzymes and may precipitate proteins (Thirman*et al.*, 1993).

Alkaloid (12.22%) was also obtained. It has diverse functions and some acts as pain relievers, anti- tumors, stimulants, etc. They also uniformly invoke a bitter taste (Rhoades, 1976). It reduces blood pressure, kills tumor cells, stimulate circulation and respiration (Makkar*et al.,* 2007). At very high level, it exerts toxicity and adverse effects, especially in physiological and neurological activities (example rapid heartbeat and paralysis) which can lead to death.

Phytate at 6.12mg/100g was obtained from the extract used during the present study. It was reported that, it has protective action in carcinogesis and also believed to be a potential antioxidant as well as prevent kidney stones (Lu *et al.,* 2004). Excessive amount of phytic acid in the diet will form

insoluble complexes with multi charged metals (Nolan *et al.*, 1987), such as Cu 11, Zinc 11, Cu 11, Ca 11 and Iron 11. This results in deficit in the absorption of some dietary mineral and lead to mineral deficiencies (Morris, 1986).

Saponin at 22.55% was also obtained as part of the phytochemicals in the extract. Research sources disclosed that saponin exert various biological benefits, such as anti-inflammatory, anti- diabetic, anti- HIV, anti- artherosclerotic and serve as protective functions like gastroprotective, hepatoprotective and hypolipidaemic (Banno*et al.*, 2004). Excess dietary saponins are highly toxic to cold blooded animals due to its haemolytic property, in which it ruptures RBC and releases haemoglobin (Sen*et al.*, 1998). They also acts as growth inhibitor due to its bitternes and throat irritating activity (Sen*et al.*, 1998).

Tannin (1.45%) was detected. Tannins have shown potential antiviral, antibacterial and anti- parasitic effects (Lu *et al.*, 2004). High level of tannin may negatively affect digestion (Kolodziej and Kiderlen., 2005).

Oxalate at 1.4 mg/ 100g was obtained. It functions as chelating agents and may chelates many toxic metals such as mercury and lead. When in excess, oxalate combines with divalent metallic cations such as Ca^{2+} to form crystals of the corresponding oxalate which are then excreted as minute crystals. The oxalate crystals can be razor sharp and may cause damage to various tissues.

Furthermore, plants harbour wide range of phytochemicals which help stimulate the immune system as well as modulate the functions of various other metabolic reactions in the body, they also acts by interfering with absorption of nutrients in the body when consumed in excess and that is why phytochemicals are referred to as two edge sword.

The results of the proximate analysis of the neem leaf aqueous extract as shown in table 2, demonstrate that, the leaf has nutritive value in that it contains moisture content, crude protein, ash content, crude fibre, carbohydrate and fat. These findings, has earlier been observed by Madaki*et al.*, (2016). However, different values has always been reported by different researchers and these could be attributed to the variations in geographical locations, methods of extraction as well as the season in which the leaves were harvested. The result revealed that the extract can serve as a good source of protein and energy. The high fibre content obtained may enhance waste elimination including bile acids, sterols and fat (Lupein, 1990). Fibre acts as an authentic broom in the intestine, absorbing toxins and carrying out harmful substances such as biliary acids, the precursor of cholesterol (Roger, 1999). It gives consistency to faeces. It has physiological effects in the gastrointestinal tract function by promoting the reduction of tracolonic pressure, which is beneficial in enteric cancer (Okwu and Emenike, 2007). The ash content (10.8%) obtained from the extract gives an indication that the extract may serve as a good source of macro- and micro- elements. The moisture content of 2.12% obtained is low when compared to the value reported by Madaki*et al.*, 2016 and this may be attributed to the variations in whether conditions and the season in which the leaves were harvested.

Moreover, based on the different vitamins and elements analyzed from the extract and their multifunctions, this extract has the potentials for providing essential nutrient for chickens. The vitamin profile as shown showed that vitamin C is the most abundant vitamin in the extract followed by vitamin B6, E, A, B1 and B2. Vitamin A promotes skeletal growth, healthy mucous membranes, healthy skin, eyes and feather. Vitamin E protects body's store of vitamin A, tissues and fat from destructive oxidation, and break down of red corpuscles, strengthens capillary walls, regulate reproductive period, prevent loss of other vitamins, aids blood flow to heart, lowers blood cholesterol and fatty acids, regulate protein and calcium metabolism. Vitamin C is essential for the formation of collagen, needed for absorption of iron, some proteins and folic acid, prevents oxidation of other vitamins, aids in metabolism of amino acids and calcium, stops internal bleeding, strengthens blood vessels, maintains hard bones and teeth, heals wounds and burns. Vitamin B6 (pyridoxine hydrochloride) aids in metabolism of protein, carbohydrate and fat, control cholesterol level, aids chemical balance between blood and tissue, prevent water retention, builds haemoglobin. Vitamin B1 (Thiamine) helps converts sugar and starch into energy, promote digestion, strengthens heart muscle, chick growth, prevent fatigue and fat deposits in arteries (Anon., 2016). Vitamin B2 (riboflavin) aids in releasing energy to body cells, enables utilisation of protein, fat and carbohydrate, essential for the formation of RBC, and builds nucleic acid (Anon., 2016).

The elemental analyses revealed the presence of calcium, phosphorus, magnesium, sodium, zinc, iron and potassium with potassium being the most abundant and zinc the least mineral component contained in the extract. Calcium builds bones, aids in proper functioning of the muscles, hearts, nerves, and iron utilisation, helps blood coagulation, regulates body temperature, aids nerve function and bone growth. It helps in the utilisation of vitamin B, C and E, promotes absorption and metabolism of other minerals, activates enzymes for metabolism of carbohydrate and amino acids and prevents calcium deposits in ureters. Iron forms a constituent of haemoglobin which carries oxygen to the tissues by blood circulation. Zinc eliminates cholesterol deposits, aids in absorption of B- vitamins, manufacture of enzymes and insulin, and metabolism of carbohydrate. It is also essential for growth, aids in healing, prevents sterility, keeps feather glossy and smooth (Anon., 2016).

Conclusively, the present findings indicates that the neem leaf extracts studied contained an important chemical compositions that are of medicinal as well as of nutritive value which when explored and utilized would be of help to the human and animal lives.

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